SARTORIUS

Application Note

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DNA Removal Using Sartobind® Q in mAb Purification

Ion Exchange Chromatography With Sartobind® Membrane Adsorbers

Introduction

Sartorius Membrane Adsorbers are very effective for rapid DNA removal at all scales. This application note describes one of the first industrial applications for membrane chromatography i the methods used to implement Sartobind membrane adsorbers in removing DNA from a monoclonal antibody (mAb) moving from the lab bench to final scale-up.

Membrane adsorbers speed up chromatography as the large pores allow convective flow and do not depend on diffusion as in traditional gel bead based chromatography. Sartobind capsules are similar to standard filter capsules, except that the adsorbing membrane is wound on a core to form a cylinder. Flow is from the top into the outside channel, crossing perpendicularly through the membrane layers and into a center channel connected to the capsule outlet. When considering a method, the following requirements should be met: First, easy transfer from lab bench to final scale is critical. A cumbersome and time consuming scale up will escalate costs. Second, fast and convenient sanitization of the process equipment simplifies the process immensely. And finally, quick and straight forward validation of the process is a must. Sartobind membrane adsorbers meet these requirements as shown in the following description of the development of a process to remove DNA at manufacturing scale.

Find out more: www.sartorius.com/en/products/process-chromatography

1. DNA Removal in Lab Scale by Anion Exchange Membrane and Resin*

1.1 Materials and Methods

Load material	Monoclonal antibody, 0.63 mg/mL, spiked with 25 µg/mL DNA isolated from CHO-cells
Buffer	10 mM phosphate / 250 mM NaCl, pH 7.25, 25 mS/cm
Device	Sartobind® Q 2.8 mL device, Q Sepharose® FF, height 11 cm, diameter 1.0 cm, bed volume 8.6 mL

A total of 900 mL mAb solution was passed through a Sartobind® Q device at a flowrate of 30 mL/min. The chromatographic column was loaded with mAb as well, however, a linear flowrate of 100 cm/h equivalent to 78 mL/h was applied, which is a magnitude below the flowrate applied to the membrane (1800 mL/h). The effluent was monitored photometrically parallel at 254 nm (DNA) and 280 nm (protein). Beside photometrical determination, 50 mL fractions were taken and DNA content was measured. A slight, continuous increase of the 254 nm value starting at 620 mL of effluent indicated DNA breakthrough.

1.2 Results

The binding capacity of Sartobind® Q was almost 10 times higher than that of Q Sepharose® FF. The process time was reduced 23-fold by Sartobind® (Table 1).

	Flow rate [mL/h]	Binding capacity for DNA [mg/mL]	Process time [h]
Sartobind® Q 2.8 mL	1,800	5.6	0.5
Q Sepharose® FF 8.6 mL	78	0.6	11.5

Table 1: The applied flow rate, the DNA binding capacity and the process time of Sartobind® Q and Q Sepharose® FF

2. DNA Removal in mAb Manufacturing Process*

Based on the results from the scale down trials, Sartobind® Q was implemented at the process scale.

2.1 Materials and Methods

Eight 2,000 L mAb batches were processed using Sartobind® Q 70 mL. Three 12,500 L mAb batches were processed using Sartobind® Q 560 mL. The data below uses the calculated average amount of DNA from the 2,000 L and 12,500 L batches.

2.2 Results

The DNA impurity was removed from the mAb to a level below the limit of detection in both the 2,000 L and 12,500 L batches (Figure 1).

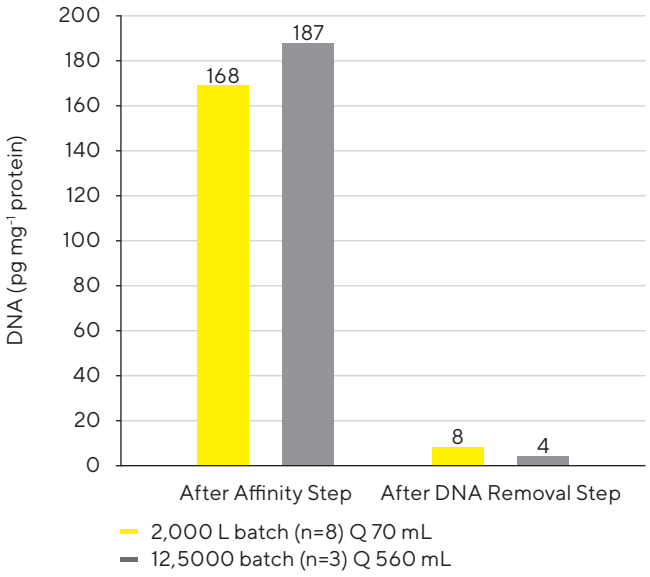

Figure 1: DNA amount [pg/mg protein] after the affinity chromatography step and after DNA removal step with Sartobind® Q 70 mL and 560 mL devices from eight 2,000 L and three 12,500 L batches, respectively.

The results in this application note have been created with previous Sartobind® Q devices. The equivalent current devices are listed on the right side in the following table.

Scale	Used devices*		Current capsules recommended**	
	Bed vol. [mL]	Bed height [mm]	Bed volume [mL]	Bed height [mm]
Lab	2.8	1.4	1 (nano)	4
Process	70	4	75	4
Process	560	4	600	4

Discussion

As demonstrated at each scale, the high flow rate achievable with Sartobind® Q results in much shorter process times than would be possible if using traditional methods. The large pores of the membrane also results in a higher dynamic binding capacity for the large DNA molecules compared to Sepharose® FF.

While the results presented above demonstrate the high performance of Sartobind® Q in removing DNA during polishing steps, there are additional cost and time-saving attributes of Sartobind® capsules not demonstrated in the final data. Sartobind® is readyto- use; simply sanitize with NaOH and wash with equilibration buffer. This is in stark contrast to the time needed to prepare slurry and pack a column and there is no risk of the matrix collapsing or forming channels. The capsule format lends itself to single use, eliminating validation of cleaning and storage methods. However, a full validation package is available should the cost structure of the process require re-use. While buffer consumption is not recorded above, experience has demonstrated that using Sartobind® uses only 5-20% of the buffer required to perform the same step using column chromatography.

Conclusions

- The high flow rates achievable with Sartobind® result in time and cost savings.
- An extremely high dynamic binding capacity makes this anion exchanger an excellent means to remove contaminant DNA.
- Validation can be streamlined; capsule design lends itself to single-use.
- Ready to use capsules are robust, and do not carry the usual-risks of column chromatography such as variations in packing quality, matrix collapse, channeling, or entrapment of air.
- Sartobind® devices come ready to use and allow quick set-up eliminating labor-intensive operations.
- Buffer consumption using Sartobind® is 5-20% that of traditional column chromatography at an equal scale.
- Full range of capsules allows seamless scale-up from laboratory bench to manufacturing process.

Reference

* Joachim K. Walter, Boehringer Ingelheim Pharma KG, Strategies and Considerations for Advanced Economy in Downstream Processing of Biopharmaceutical Proteins, in: Bioseparation and Bioprocessing; G. Subramanian, (Ed.), Processing, Quality and Characterization, Economics, Safety and Hygiene, Wiley VCH, 1998, vol. II, pp. 447-460, and personal communication.

** Sartorius data sheet: Sartobind® Q and S, 4 and 8 mm bed height, order number: 85034-535-76

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